mM sodium borate, about 1 M to about 3 M sodium chloride, about 5% to about 20% ethanol, and about 0.005 mM to about 10 mM dithiothreitol.

24. (New) The method of claim 23 wherein the denaturation buffer has a pH of about 8.5 to about 10 and comprises about 2 M urea, about 5 mM sodium borate, about 1.5 M sodium chloride, about 15% ethanol, and about 0.2 mM dithiothreitol.

25. (New) The method of claim 24 wherein the denaturation buffer has a pH of about 9.0 to about 9.5.

26. (New) The method of claim 25 wherein the denaturing and renaturing are performed together and conducted for about 15 hours to about 20 hours at room temperature.

REMARKS

Claims 1-21 are pending in the present application. Claim 1 has been amended to correct a typographical error. New claims 22-26 have been added, support for which can be found throughout the specification and at, for example, page 8, lines 19-21, and page 12, line 22 to page 13, line 15 of the specification. No new matter has been added. Upon entry of the present amendment, claims 1-26 will be pending.

As a preliminary matter, Applicants acknowledge receipt of the "Attachment for PTO-948" outlining changes for prosecution of applications containing drawings. To date, however, no Form PTO-948 has been received. Accordingly, the "Attachment for PTO-948" is not relevant in the present application.

In addition, as outlined in the Power of Attorney With Revocation filed April 11, 2001, please address all further correspondence in this application to:

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I. The Claims Are Clear And Definite

Claims 1-21 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. The Office Action asserts that the phrases "a substantial amount" in step (b), "a significant proportion" in steps (f), (i), (l) and (m) and "a substantial proportion" in step (l) are vague and indefinite because there is no support or guidance in the specification that would allow the skilled artisan to determine what is within the scope of the claim. Applicants traverse the rejection and respectfully request reconsideration because the claims are clear and definite.

Persons of ordinary skill would, for example, have no difficulty in determining whether a particular amount of adsorbed non-IGF-I material (in step (b)) or a particular amount of non-authentic IGF-I (in step (l)) is substantial. Indeed, Applicants provide herewith a copy of a definition from *The American Heritage Dictionary* in which "substantial" is defined as "[c]onsiderable in ...amount." The Office Action provides no reasoning or evidence that one skilled in the art, having examined Applicants' specification, would not be able to determine whether a particular amount or proportion is "substantial." Applicants also enclose a definition from *The American Heritage Dictionary* in which "significant" is defined as "[N]otable; valuable." The Office Action provides no reasoning or evidence that one skilled in the art, having examined Applicants' specification, would not be able to determine whether a particular proportion is "significant." The claims are definite within the meaning of § 112. *In re Mercier*, 185 U.S.P.Q. 774 (C.C.P.A. 1975) (claims sufficiently define an invention so long as one skilled in the art can determine what subject matter is or is not within the scope of the claims). Thus, claims 1-21 are clear and definite. Accordingly, Applicants respectfully request that the rejection of claims 1-21 under 35 U.S.C. § 112, second paragraph be withdrawn.

II. The Claimed Invention Is Not Obvious

Claims 1-21 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over U.S. Patent No. 5,231,178 (hereinafter, the "Holtz reference") in combination with Hart *et al.*, *Biotechnol. Appl. Biochem.*, **1994**, *20*, 217-232 (hereinafter, the "Hart reference"), U.S. Patent No. 5,288,931

(hereinafter, the "Chang reference"), Sofer *et al.*, *Biotech.*, **1983**, *November/December*, 198-203 (hereinafter, the "Sofer reference"), and U.S. Patent No. 5,459,052 (hereinafter, the "Skriver reference"). The Office Action mistakenly asserts that it would have been *prima facie* obvious for one skilled in the art to have modified the method reported in the Holtz reference with the addition of an unfolding/folding step as allegedly reported in the Chang and Hart references. The Office Action mistakenly asserts that it would have been *prima facie* obvious for one skilled in the art to have replaced the final gel filtration step of the Holtz reference with the reverse-phase HPLC step the the Skriver reference. Applicants traverse the rejection and respectfully request reconsideration because there is no motivation to combine the cited references and, even if combined, the claimed invention would not be produced.

A critical step in analyzing the patentability of claims pursuant to § 103(a) is casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field." *In re Kotzab*, 217 F.3d 1365, 1369, 55 U.S.P.Q.2d 1313, 1316 (Fed. Cir. 2000). "The invention must be viewed not with the blueprint drawn by the inventor, but in the state of the art that existed at the time." *In re Dembiczak,* 175 F.3d 994, 999, 50 U.S.P.Q.2d 1614, 1617 (Fed. Cir. 1999) (quoting *Interconnect Planning Corp. v. Feil*, 774 F. 2d 1132, 1138, 227 U.S.P.Q. 543, 547 (Fed. Cir. 1985). To establish a *prima facie* case of obviousness, "there must be some teaching, suggestion or motivation in the prior art to make the specific combination that was made by the applicant." *In re Dance*, 160 F.3d 1339, 1343, 48 U.S.P.Q.2d 1635, 1637 (Fed. Cir. 1998). "In other words, the examiner must show reasons that the skilled artisan, confronted with the same problem as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed." *In re Rouffet*, 149 F.3d 1350, 1357, 47 U.S.P.Q.2d 1453, 1458 (Fed. Cir. 1998). The Office Action has failed to comply with each of these requirements and, thus, has failed to establish a *prima facie* case of obviousness.

The methods reported in the Holtz reference could not be successfully modified by adding the refolding step of the Chang or Hart references and the reverse phase step of the Skriver reference, as purported in the Office Action, to arrive at Applicants' claimed invention. The objective of the

Holtz reference is to ***remove*** impurities with the cation exchange step, including ***multimeric and/or misfolded forms of IGF-I***, rather than retain them fort a subsequent refolding step. To carry this out, the Holtz reference reports using at least two washes in the first cation exchange step (a dilute weak acid followed by a dilute weak acid at higher ionic strength -- sodium acetate and sodium chloride; column 10, lines 25-47 of the Holtz reference). Systems such as the one reported in the Holtz reference, in fact, ***remove*** aberrant forms of IGF-I that are less tightly bound to the cation exchange resin than is authentic IGF-I (see, page 11, lines 7-18 of Applicants' specification). This is in complete contravention to the present invention which is particularly designed to ***retain*** both authentic and non-authentic forms of IGF-I on the cation exchange matrix so that the aberrant IGF-I forms can be refolded in the unfolding/refolding step to increase the ultimate yield of authentic IGF-I. Thus, modification of the Holtz process as proposed in the Office Action ***would not*** result in Applicants' claimed invention because there would be no aberrant IGF-I species to refold and subsequently subject to reverse phase chromatography.

Further, a § 103 rejection based upon a modification of a reference that destroys the intent, purpose, or function of the invention disclosed in the reference is not proper and a *prima facie* case of obviousness cannot therefore be made. In short, there is no technological motivation for engaging in the modification of the Holtz reference to encompass Applicants' claimed invention. *See, In re Gordon*, 733 F.2d 900, 221 U.S.P.Q. 1125 (Fed. Cir. 1984) (to render the prior art inoperable for its intended purpose is the antithesis of obviousness). Thus, there is no motivation to modify the Holtz reference as suggested in the Office Action, nor would there be a reasonable expectation of success. Indeed, there is every reason to believe that it would not be a success.

The secondary reference fail to cure the deficiencies of the Holtz reference. The Chang and Hart references are directed solely to refolding IGF-I that has been expressed in *E. coli* as refractile inclusion bodies precipitated, for example, in the periplasmic space (see, column 9, lines 49-51 of the Chang reference). Such inclusion bodies include IGF-I molecules (both aberrant and authentic) in an insoluble, inactive form (see, for example, column 8, lines 63-65 of the Chang reference and page 218, under Materials and Methods of the Hart reference). This is also inapposite to the present invention which is directed to purifying largely soluble IGF-I from a medium.

It is also well known in the art that the particular purification method used to isolate a given protein is largely dependent on the physical form of the protein being purified. The physical form of IGF-I species present in a cell medium would be expected to be different than insoluble IGF-I species found in inclusion bodies packaged in the periplasmic space. Thus, the purification of IGF-I from *E. coli* poses very different problems than the purification of IGF-I from medium.

The Chang and Hart references therefore focus on problems caused by the use of bacterial expression systems such as *E. coli* -- the recovery of an active protein from an insoluble inclusion body. It is only with the benefit of Applicants' specification that the Office Action can assert that the teachings of the Chang and Hart references could be successfully combined with the remaining reference to produce Applicants' claimed invention. Such hindsight reconstruction of the invention cannot suffice to support a *prima facia* showing of obviousness. *In re Fine*, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988) ("One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention."). Applicants further note that "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed. Cir. 1992). Under this standard, none of the prior art of record, alone or in any proper combination, discloses or suggests the present invention. This is not to say that it is impossible to combine selected elements of several references to show the obviousness of an invention, however, there still must be a "suggestion or motivation in the prior art to make the selection." *In re Gorman*, 18 U.S.P.Q.2d 1885, 1888 (Fed. Cir. 1991) (claim held obvious in view of combined teachings of references showing elements for same purpose as claimed invention).

Finally, the Skriver reference (cited for teaching the use of reverse phase HPLC) and the Sofer reference (cited for allegedly teaching optimal chromatography purification schemes for proteins) also do not cure the deficiencies of the Holtz reference. As with the Chang reference, the Skriver reference pertains to methods for increasing yields of IGF-I purified from inclusion bodies formed by expression of the protein in *E. coli* (see, column 2, lines 48-50 of the Skriver reference). To enhance IGF-I yields, the Skriver reference produces an amino terminally-extended IGF-I molecule, termed "Ala-Glu-IGF-I." The Ala-Glu extension is ultimately cleaved from the remainder

of the molecule using dipeptidyl amino peptidase (DAP-1). As described in Example 9, column 13, line 66 of the Skriver reference, the reverse-phase HPLC step is done in part to terminate the enzymatic reaction used to cleave the Ala-Glu portion of the molecule. Thus, the HPLC step in the Skriver reference is added to solve a problem peculiar to the Skriver reference -- *i.e.*, stopping the enzymatic reaction used to cleave the amino terminally extended IGF-I molecule reported therein. There is no suggestion to extend this step to a purification scheme as recited in Applicants' claims.

Further, the Sofer reference is a general reference pertaining to optimizing purification schemes for proteins. The Sofer reference fails to directly address IGF-I. The Sofer reference is cited in the Office Action as suggesting the use of "high-resolving techniques, including HPLC, for increased resolution as well as reduced separation time." Office Action, page 4. Applicants do not dispute that HPLC is and was a well known method for enhancing protein resolution. There is absolutely no suggestion in the Sofer reference, however, to use a reverse-phase step in a multistep purification method adapted to address the particular problems inherent in the recombinat production of IGF-I.

The Office Action appears to be viewing each step of the multistep process alone, independent of the process as a whole. It is axiomatic that obviousness must be viewed in the context of the invention ***as a whole***, rather than by focusing on individual elements of the invention. Indeed, as stated by the court in *Hybritech v. Monoclonal Antibodies*, 231 U.S.P.Q. 81, 83 (Fed. Cir. 1986):

> Focusing on the obviousness of substitutions and differences instead of on the invention as a whole, as the district court did in frequently describing the claimed invention as the mere substitution of monoclonal for polyclonal antibodies in a sandwich assay, was a ***legally improper*** way to simplify the difficult determination of obviousness.

See, also, In re Wright, 6 U.S.P.Q.2d 1959, 1961 (Fed. Cir. 1988) (it is the invention as a whole that must be considered in obviousness determinations.").

As discussed above, the Holtz reference (the primary reference) does not even lend itself to the unique combination of steps claimed in the present application because practicing the Holtz methodology, even in combination with the teachings of the secondary references, would not achieve Applicants' results. Therefore, when one correctly focuses on the invention as a whole rather than on

individual steps, it is clear that the cited combination of references cannot stand. Thus, the claimed invention is not obvious in view of the combination of cited references. Accordingly, Applicants respectfully request that the rejection of claims 1-21 under 35 U.S.C. § 103(a) be withdrawn.

Additionally, new claims 22-26, directed to methods for refolding IGF-I derived from a yeast cell medium, are patentable over the applied references. As discussed above, the references cited in the Office Action pertaining to refolding IGF-I are directed to refolding a protein derived from an *E. coli* expression system. Such systems produce IGF-I in the form of insoluble inclusion bodies. The new refolding claims of the present application, on the other hand, pertain to methods performed on IGF-I that has been secreted into a yeast cell medium. It is well known in the art that the choice of a particular host cell, for example, a yeast host versus a bacterial host, has a significant impact on the final form and yield of the protein products. This, in turn, affects the particular methods used to manipulate the protein product.

Further, *E. coli* lacks eukaryotic glycosylation systems while yeast can carry out post-translational modification of proteins. Additionally, yeast systems have different proteases than those found in *E. coli* expression systems. Thus, the production of IGF-I in a yeast host would be expected to result in different IGF-I variants, including different clipped and glycosylated species, than those recombinantly produced in a bacterial host. Therefore, no analogy with respect to refolding procedures from one system to the other system can be correctly made. Accordingly, the new refolding claims are distinguished over the applied references.

III. There Is No Double Patenting

Claims 1-21 are rejected under the doctrine of double patenting over claims 1-21 of U.S. Patent No. 5,650,496 and claims 1-25 of U.S. Patent No. 6,207,806. Applicants traverse the rejection and respectfully request reconsideration thereof because there is no double patenting.

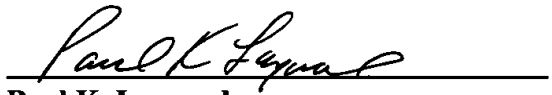
Claims 1-21, as well as new claims 22-26, of the present application are not directed to the "same invention" as that of claims 1-21 of U.S. Patent No. 5,650,496 or claims 1-25 of U.S. Patent No. 6,207,806. "Same invention" means ***identical*** subject matter. *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1984); *In re Ockert*, 114 U.S.P.Q. 330 (C.C.P.A. 1957); and *In re Vogel*, 164

U.S.P.Q. 619 (C.C.P.A. 1970). As stated in § 804 of the M.P.E.P., if there is an embodiment of the invention that falls within the scope of one claim but not the other, then "identical subject matter **is not** defined by both claims and statutory double patenting would not exist" (emphasis added). Because the scope of the claims recited in the present application and U.S. Patent No. 5,650,496 or U.S. Patent No. 6,207,806 differ, statutory double patenting is precluded. Accordingly, Applicants respectfully request that the double patenting rejection be withdrawn.

IV. Conclusion

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 564-8906 if there are any questions regarding Applicants' claimed invention. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,



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Date: **December 18, 2001**

Enclosure

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claim 1 has been amended as follows:

1. (Amended) A process for purifying correctly folded monomeric insulin-like growth factor-I (IGF-I) from a medium containing IGF-I peptides, comprising the steps of:

(a) contacting the medium with a sufficient quantity of a first cation exchange matrix under conditions allowing adsorption of at least about 95% of total IGF-I from the medium;

(b) washing the IGF-I-loaded first cation exchange matrix with a first cation exchange wash buffer, which removes a substantial amount of adsorbed non-IGF-I material without removing a substantial amount of authentic or non-authentic IGF-I;

(c) eluting all forms of adsorbed IGF-I from the cation exchange matrix of step (a) by contacting said cation exchange matrix with a sufficient quantity of a first cation exchange elution buffer, which has a sufficiently high pH or ionic strength to displace substantially all of said authentic and non-authentic IGF-I from said cation exchange matrix;

(d) transferring the IGF-I-containing eluate from step (c) into an unfolding/refolding buffer, which:

(i) reduces the intrachain disulfide bonds of IGF-I protein and promotes unfolding without permanent denaturation; and

(ii) permits refolding of the IGF-I and reoxidation to form properly-paired intrachain disulfide bonds;

(e) contacting the properly folded IGF-I from step (d), after transfer into a suitable solvent system, with a sufficient quantity of a hydrophobic interaction chromatography matrix under conditions allowing adsorption of at least about 95% of said IGF-I from said eluate;

(f) washing the IGF-I-loaded hydrophobic interaction chromatography matrix with a hydrophobic interaction wash buffer having an ionic strength sufficiently low to remove most of the non-authentic IGF-I, but not so low as to remove a significant proportion of the authentic IGF-I from the hydrophobic interaction chromatography matrix;

(g) eluting the adsorbed IGF-I from said hydrophobic interaction chromatography matrix by contacting said matrix with a hydrophobic interaction elution buffer, which has a sufficiently elevated pH, or sufficiently low ionic strength, to cause displacement of substantially all of the adsorbed authentic IGF-I from said matrix;

(h) contacting the eluate from step (g) with a sufficient quantity of a second cation exchange matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(i) washing the IGF-I-loaded second cation exchange matrix with a cation exchange wash buffer having a sufficiently high ionic strength, or sufficiently high pH, to remove a significant proportion of non-authentic IGF-I, but not so high as to remove a significant proportion of authentic IGF-I;

(j) eluting the adsorbed IGF-I from said second cation exchange matrix by contacting said matrix with a second cation exchange elution buffer, which has a sufficiently high ionic strength, or sufficiently high pH, to displace substantially all of the adsorbed authentic IGF-I from said matrix;

(k) contacting the eluate from step (j), in an aqueous buffer, with a suitable quantity of a reverse phase chromatography matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(l) washing the IGF-I-loaded reverse phase chromatography matrix with an aqueous/organic reverse phase wash buffer having an organic solvent concentration sufficiently high to remove a substantial proportion of non-authentic IGF-I, but not so high as to remove a significant proportion of authentic IGF-I; and

(m) eluting the adsorbed IGF-I from said reverse phase chromatography matrix with an aqueous/organic buffer having an organic solvent concentration high enough to remove substantially all of the authentic IGF-I without removing a significant proportion of multimeric forms of IGF-I.

New claims 22-26 have been added as follows.

22. (New) A method for refolding an insulin-like growth factor-I (IGF-I) polypeptide derived from a yeast cell medium to yield an authentic, properly folded IGF-I polypeptide comprising

denaturing and renaturing IGF-I species present in an IGF-I mixture from said yeast cell medium using a denaturation buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient amounts and under conditions that allow for the reduction and subsequent oxidation of disulfide bonds, thereby producing an authentic, properly folded IGF-I polypeptide.

23. (New) The method of claim 22 wherein the denaturing and renaturing are performed together using a denaturation buffer comprising about 1.5 to about 3 M urea, about 1 mM to about 15 mM sodium borate, about 1 M to about 3 M sodium chloride, about 5% to about 20% ethanol, and about 0.005 mM to about 10 mM dithiothreitol.

24. (New) The method of claim 23 wherein the denaturation buffer has a pH of about 8.5 to about 10 and comprises about 2 M urea, about 5 mM sodium borate, about 1.5 M sodium chloride, about 15% ethanol, and about 0.2 mM dithiothreitol.

25. (New) The method of claim 24 wherein the denaturation buffer has a pH of about 9.0 to about 9.5.

26. (New) The method of claim 25 wherein the denaturing and renaturing are performed together and conducted for about 15 hours to about 20 hours at room temperature.